

University of Groningen

## Cellular localization of choline-utilization proteins in *Streptococcus pneumoniae* using novel fluorescent reporter systems

Eberhardt, Alice; Wu, Ling J.; Errington, Jeff; Vollmer, Waldemar; Veening, Jan-Willem

*Published in:*  
Molecular Microbiology

*DOI:*  
[10.1111/j.1365-2958.2009.06872.x](https://doi.org/10.1111/j.1365-2958.2009.06872.x)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2009

[Link to publication in University of Groningen/UMCG research database](#)

### *Citation for published version (APA):*

Eberhardt, A., Wu, L. J., Errington, J., Vollmer, W., & Veening, J-W. (2009). Cellular localization of choline-utilization proteins in *Streptococcus pneumoniae* using novel fluorescent reporter systems. *Molecular Microbiology*, 74(2), 395-408. <https://doi.org/10.1111/j.1365-2958.2009.06872.x>

### **Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### **Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Eberhardt et al, supplementary table.

**Table S1.** Oligonucleotides used in this study.

Oligonucleotide	Sequence (restriction site in bold)	Restriction site	Used for (plasmid or construct)
gfp- F+BglII_RBS_stre p	GCGC <b>AGATCT</b> TAAGGAGGCAAATATG AAACATCTTAGCAAAGGAGAAGAACTT TTCAGTGGAGTTGTCC GCGC <b>GCTCAGCT</b> TATTATGCGGCCGC TCCACTAGTTCCAGCTTTAGCTGCAGC	<i>BglII</i>	pJWV25
gfp-R+BlpI	TTCTCCACC AGTATC <b>CCTGCAGGA</b> AGTTAGCTAATA	<i>BlpI</i>	pJWV25
<i>tarI</i> +PtarI-F( <i>SbfI</i> )	AGAAATGAAGGGC	<i>SbfI</i>	pAE03-tarI
<i>tarI</i> +hingeregion- R( <i>NheI</i> )	CAGTAC <b>GCTAGC</b> AGCAGAACCGTCTT TCTCAATCATACTTTTTGC CGCG <b>GAATTC</b> CAATGACATCATCATC ATTG	<i>NheI</i>	pAE03-tarI
<i>licC</i> -F( <i>EcoRI</i> )	GCGC <b>GCTAGCT</b> CCACCAGATCCATTT TCGTTTTTAAGAATTTCTTCTAATTTAC	<i>EcoRI</i>	pAE03-licC
<i>licC</i> -R( <i>NheI</i> )	GATAGTCTTGG CGCG <b>GAATTC</b> GGGACGACGATATTGA	<i>NheI</i>	pAE03-licC
<i>licD1</i> -F( <i>EcoRI</i> )	TCTGTCC GCGC <b>GCTAGCT</b> CCACCAGATCCATCC TCCAATTTATAAGCGTGAAATTCATGA C	<i>EcoRI</i>	pAE03-licD1
<i>LicD1</i> -R( <i>NheI</i> )	TACTAC <b>GAATTC</b> GTTATGGAACCATGC	<i>NheI</i>	pAE03-licD1
<i>licD2</i> -F( <i>EcoRI</i> )	TTGGAGCC	<i>EcoRI</i>	pAE03-licD2
<i>licD2</i> +hingeregion- R( <i>NheI</i> )	GCTGAC <b>GCTAGC</b> AGCGCTACCGTTTT TGCGATAAGCTTTAATGC	<i>NheI</i>	pAE03-licD2
<i>tarI</i> -promoter- F( <i>SbfI</i> )	CATCAC <b>CCTGCAGG</b> TATATCATAAAGT TAGCTAATAAG	<i>SbfI</i>	<i>PtarI</i>
<i>tarI</i> -promoter- R( <i>Bam</i> HI)	CATCAT <b>GGATCC</b> CTATTTTCTCCTTTG TCTTAC CCACAT <b>GGATCC</b> GTGGAGAAAATCAT TAAAG	<i>Bam</i> HI	<i>PtarI</i> pAE03- PtarI-licA
<i>licA</i> -F( <i>Bam</i> HI)	ACTACT <b>GCTAGCT</b> CCACCAGATCCCTT TTCATCTGAACCTCCATAAGAAGCCAA ACC	<i>Bam</i> HI	pAE03- PtarI-licA
<i>licA</i> +hingeregion- R( <i>NheI</i> )	CCACAT <b>GGATCC</b> ATGATTAATCAAATT TATCAAC	<i>NheI</i>	pAE03- PtarI-licA
<i>tarJ</i> -F( <i>Bam</i> HI)	GATGAT <b>GAATTC</b> AGAACCGCTTACTTC	<i>Bam</i> HI	pAE03- PtarI-tarJ
<i>tarJ</i> +hingeregion- R( <i>NheI</i> )	CCACTTAAACACTGTTTTAAAGGC GCGC <b>ACTAGT</b> AAAAGTATAAAATTTAA TGCTCTATCTTACATGGG	<i>NheI</i>	pAE03- PtarI-tarJ
<i>tacF</i> -F( <i>SpeI</i> )	CGAT <b>GCGGCCGCT</b> TATTACTATGATTT TTTAAATTTATTTTAAAGGCTAGGACA TGG	<i>SpeI</i>	pJWV25- tacF
<i>tacF</i> -R( <i>NotI</i> )	GCGC <b>ACTAGT</b> GAGGTTTACAGATGAAAA GTAAAAACGG	<i>NotI</i>	pJWV25- tacF
<i>licB</i> -F( <i>SpeI</i> )	CGAT <b>GCGGCCGCT</b> TATTATTCTTTAAT	<i>SpeI</i>	pJWV25- licB
<i>licB</i> -R( <i>NotI</i> )	AATAATATAAACTCCAGCAATGACG GCGC <b>ACTAGT</b> AGGAATAGAATGCCAA TTACATC	<i>NotI</i>	pJWV25- licB
<i>divIVA</i> -F( <i>SpeI</i> )	CGAT <b>GCGGCCGCT</b> TATTACTTCGTGTT CTTCATACATTGGGC	<i>SpeI</i>	pJWV25- divIVA
<i>divIVA</i> -R( <i>NotI</i> )		<i>NotI</i>	pJWV25- divIVA

<i>licB</i> -up-F	GGTTGCAGCCATTACAGAGTTTGTC GCGC <b>GGATCC</b> TTACTTTTCATCTGAAC	none	KO of <i>licB</i>
<i>licB</i> -up-R( <i>Bam</i> HI)	CTCCATAAGAAGCC	<i>Bam</i> HI	KO of <i>licB</i>
<i>licB</i> -down-F( <i>Bam</i> HI)	GCGC <b>GGATCC</b> TAAAGGAGATTCTGT GAAAGCCATCATCTTAGC	<i>Bam</i> HI	KO of <i>licB</i>
<i>licB</i> -down-R	CCAGTCCTGTTCCAATCACTGCAATG G	none	KO of <i>licB</i>
<i>ermB</i> -F( <i>Bam</i> HI)	GCGC <b>GGATCC</b> CTTAAGAGTGTGTTGA TAGTGCAGTATC	<i>Bam</i> HI	KO of <i>licB</i>
<i>ermB</i> -R( <i>Bam</i> HI)	GCGC <b>GGATCC</b> TTATTTCTCCCGTTAA ATAATAGATAAC	<i>Bam</i> HI	KO of <i>licB</i>
pAE03-screen F	ATTACGCCAAGCTTGCATGC	none	pAE03 screening
pAE03-screen R	ACAAGAATTGGGACAACTCC	none	pAE03 screening
pJWV25-screen F	GACAACCATTACCTGTGCGAC	none	pJWV25 screening
pJWV25-screen R	TTGGTGCAAGGAAGGTCATC	none	pJWV25 screening confirming insertion of pJWV25 in
<i>bgaA</i> -check-F	CCACTCGCAACAATCACTTGG	none	<i>bgaA</i> confirming insertion of pJWV25 in
<i>bgaA</i> -check-R	GGTTGAGAACTATTTTGTTCC	none	<i>bgaA</i> confirming presence of
check-for- <i>ermB</i> -F	AAAGGGCATTTAACGACGAACTGG	none	<i>ermB</i> confirming presence of
check-for- <i>ermB</i> -R	GACAATACTTGCTCATAAGTAACGG	none	<i>ermB</i> confirming correct
check-for-pAE03- <i>licD1</i> -F	TCATCCACTTGGGACATATC	none	integration confirming correct
check-for-pAE03- <i>licD1</i> -R	ATCAATATAGTCCAGCAGGGC	none	integration confirming correct
check-for-pAE03- <i>licD2</i> -F	GATTATATGACCCTACCACC	none	integration confirming correct
check-for-pAE03- <i>licD2</i> -R	GTAGTTTGTATAATCCCCCTC	none	integration confirming correct
check-for-pAE03- <i>tarI</i> -F	ACGAATTCCCATGTAAGATAG	none	integration confirming correct
check-for-pAE03- <i>tarI</i> -R	CTTGGTCAATAGCCTCTTCCTG	none	integration confirming correct
check-for-pAE03- <i>P<sub>tarI</sub>tarJ</i> -F	GAAAAGATGTGGCTTTGGCC	none	integration confirming correct
check-for-pAE03- <i>P<sub>tarI</sub>tarJ</i> -R	ACACTGAGGACTTCCTCTTC	none	integration confirming correct

check-for-pAE03- <i>P<sub>tar</sub></i> <i>licA</i> -F	TTCATCGTGTCTTTGCAACC	none	integration confirming correct integration confirming
check-for-pAE03- <i>P<sub>tar</sub></i> <i>licA</i> -R	AAAATACCTGAGAGAAGGCC	none	correct integration confirming
check-for-pAE03- <i>licC</i> -F	GACGTCACTTGTCGTCATTGC	none	correct integration confirming
check for pAE03- <i>licC</i> -R	TCACCCAACAAAAGCTCCTG	none	correct integration

---